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Rooting the phylogenetic tree for winged insects: independent adaptations to terrestrial life

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ABSTRACT

Although numerous articles have been published on insect phylogeny using a great variety of techniques, there is no consensus on the nature of the first winged insects, the ancestors of holometabolous insects or the causes for the origin of metamorphosis. This discord has resulted in the lack of secure foundations within entomological theory. However, several recent articles provide key information which may help to resolve some of the long-standing disputes. Some biologists have argued that the first winged insects might have been amphibiotic rather than terrestrial and that metamorphosis might have originated as an adaptation to amphibiotic life. Thus entomological theory may now be passing through a paradigm shift where, for the first time, the phylogenetic tree for all insects may be firmly rooted.

KEY WORDS: Adaptation, ancestors, aquatic, entomology, insect, metamorphosis, paradigms, phylogeny, terrestrial, wings.

INTRODUCTION

Ideas about the origin and evolution of insects can be traced back to Aristotle, but entomologists still do not understand the interrelationships of insect orders and have not reached consensus on the origin and appearance of the first holometabolans (Bitsch *et al.* 2004). One of the greatest controversies in entomology concerns the rooting of the phylogenetic tree for winged insects, and the stems of the different orders. Without knowledge of the ground plan of a taxon there is no basis for objective comparison with other taxa (Willmann 1997). Although DNA sequence data are now available from most major insect orders (Giribet & Ribera 2000; Whiting 2002; Bitsch *et al.* 2004), there has been a lack of coordination of studies and it has been said that the whole of our efforts is little greater than the sum of its parts (Caterino *et al.* 2000). Although cladistics or phylogenetic systematics has helped to sort out many relationships, it also has inherent limitations and is not capable of resolving ancestor-descendant relationships because these appear as sister groups that originated simultaneously (Krell & Cranston 2004). This suggests that Lepidoptera and Trichoptera are equally ancient, even though Trichoptera are known from much earlier in the fossil record (Carpenter 1992). This leads to the conclusion that there is a hypothetical gap in the fossil record of Lepidoptera (Toms 1985). If Lepidoptera and Trichoptera are seen as sister groups, this presupposes a concept of evolution and it is therefore reasonable to investigate the nature of the hypothetical ancestor of each group. Ultimately, there should be agreement between morphology, molecular phylogenetics, developmental biology and theoretical biology. Unfortunately, the hypothetico-deductive method has not been fully utilized, and thus key issues have been ignored. For example, theoretical entomology suggests that the amphibiotic orders Ephemeroptera, Odonata, Plecoptera, Megaloptera and Trichoptera may be critical orders in the evolution of insects, yet they have been omitted from many molecular studies (e.g. Caterino *et al.* 2000).

Most hypotheses about the origin and evolution of insects can be accommodated in one of two opposing models (Table 1), an aquatic model (Toms 1984*a*, 1986) or a terrestrial model (Little 1990; Pritchard *et al.* 1993). A compromise between these models has also been proposed (e.g. Kukalová-Peck 1978). A solution to this dispute could have a dramatic impact on entomological theory, especially if it is resolved in favour of the aquatic model, as the terrestrial model has been considered as one of the dominant paradigms of the last century.

The choice of a root has profound effects on the major branches of the phylogenetic tree. In the terrestrial model, the ancestors may have resembled cockroaches which are known to exist early in the fossil record (Carpenter 1992). The choice of a terrestrial candidate for the ancestor of all winged insects is a matter which needs to be resolved by those who support the terrestrial model. This insect may have played a crucial role in the evolution of winged insects. In the terrestrial model it is accepted that all aquatic insects are secondarily aquatic. If this is the case, numerous independent adaptations to aquatic life occurred. Although the terrestrial model received the majority of support in the last century, and most research has been channelled in this direction (Little 1990; Truman & Riddiford 1999), progress in substantiating such a theory has been disappointing. In fact, Caterino *et al.* referred to the state of insect molecular systematics in 2000 as a 'thriving Tower of Babel'. Since then, some progress has been made (Wheeler *et al.* 2001; Bitsch *et al.* 2004), but there is still no generally accepted hypothetical (or real) ancestor for the winged insects or the holometabolous insects, and no generally accepted explanation for the origin of wings and metamorphosis.

In contrast, according to the aquatic model, the ancestors of insects were Crustacea (Hansen 1893; Crampton 1918; Regier & Shultz 1997; Shcherbakov 1999) and the ancestors of winged insects resembled primitive mayfly larvae (Gegenbaur 1874; Toms 1984*a*). According to this model the ancestors of winged insects were aquatic and the first winged insects were amphibiotic, with aquatic larvae and terrestrial adult stages. In the aquatic model, metamorphosis originated as an inevitable consequence of an amphibiotic life cycle, with the adults adapting to terrestrial life while the larvae remained aquatic. In amphibiotic insects such as Odonata or Ephemeroptera, the aquatic larval stages and terrestrial adults are different and the changes occur with a change in habitat at metamorphosis. It is thought that an aquatic life-style was an initial characteristic of larvae of the amphibiotic orders Ephemeroptera, Odonata, Plecoptera, Megaloptera and Trichoptera. Accordingly, the larval stages of insects became terrestrial independently on several occasions. The most important plesiomorphic characters are those relating to the aquatic life of the juvenile stages, and some of the major branches of the phylogenetic tree should comprise the primitive amphibiotic orders. Many important apomorphic characters are associated with adaptations to terrestrial life in larvae. Despite these considerations, the aquatic model has not been fully explored. Nevertheless, a great deal of progress has been made recently and we may now be moving towards a paradigm shift where the phylogenetic tree for all insects could be firmly rooted for the first time.

Another possibility is a compromise between the aquatic and terrestrial models as suggested by Kukalová-Peck (1978). Starting from a distant aquatic ancestor (all terrestrial arthropods must have evolved from an aquatic ancestor at some time), it is suggested that the ancestors of winged insects left the water and then returned to water

TABLE 1

Some of the major differences between the Aquatic Model and the Terrestrial Model presented. approximately in chronological order.

The Aquatic Model	The Terrestrial Model
Insect ancestors were aquatic, related to primitive Crustacea.	Insect ancestors were terrestrial, related to primitive Myriapoda.
Ancestors of winged insects (Pterygota) were aquatic and resembled Ephemeroptera (mayfly) larvae.	Ancestors of Pterygota were terrestrial and resembled Zygentoma (silverfish).
The first Pterygota were amphibiotic, with aquatic larvae and terrestrial adults.	The first Pterygota were terrestrial in all life stages.
Arthropod tracheal respiratory systems developed several times. In early Pterygota they developed in the terrestrial adult stage.	Tracheal respiratory systems of all insects were derived from unknown terrestrial arthropod ancestors.
Wings are equivalent to gills of mayfly larvae, and evolved from existing ancient organs that can be traced back to Crustacea.	Insect wings are new developments which originated as paranotal lobes and were first used for gliding.
Holometabolous larval legs and prolegs had a common origin with the legs of adult insects.	Holometabolous larval legs and prolegs are new developments and are not equivalent to adult legs.
The most primitive living Pterygota are Ephemeroptera.	The most primitive living Pterygota may be Blattodea.
Metamorphosis originated as an inevitable adaptation to an amphibiotic life style.	Metamorphosis originated in a terrestrial hemimetabolan or early holometabolan as an adaptation enabling resource partitioning.
Terrestrial hemimetabolous insects such as Blattodea, Orthoptera and Hemiptera evolved from amphibiotic ancestors like Ephemeroptera and Plecoptera.	Amphibiotic orders such as Ephemeroptera, Odonata and Plecoptera evolved from terrestrial ancestors like Blattodea.
Amphibiotic holometabolous insects such as Megaloptera and Trichoptera evolved from amphibiotic ancestors, which may or may not have been hemimetabolous.	Amphibiotic holometabolous insects such as Megaloptera and Trichoptera evolved from terrestrial holometabolous insects.
Terrestrial holometabolous insects such as Coleoptera, Lepidoptera and Diptera evolved from amphibiotic holometabolous insects such as Megaloptera and Trichoptera.	Terrestrial holometabolous insects evolved from terrestrial hemimetabolous insects and the aquatic larvae of Megaloptera and Trichoptera are secondarily aquatic.

again to give rise to Ephemeroptera, Odonata and Plecoptera. Once this had occured, complete metamorphosis could have originated in amphibiotic insects, as suggested in the aquatic model. Eventually it may be necessary to accept that the ancestors of Ephemeroptera were terrestrial, but there is still no compelling evidence for this entirely hypothetical terrestrial stage (Sinitshenkova 2002; Zherikhin 2002). Ephemeroptera are the most primitive winged insects (Engel & Grimaldi 2004), but all living examples have aquatic larvae and there is no evidence that the first Ephemeroptera had terrestrial larvae. Before metamorphosis evolved, one set of genes presumably determined the morphology of the larvae and the adults, so both aquatic and terrestrial stages of an amphibiotic insect would have been adversely affected by this limitation until metamorphosis and/or phase-specific genes evolved (Toms 1984*b*). The recently discovered oldest mandible of a winged insect may suggest that the winged insects are 80 million years older than previously thought (Engel & Grimaldi 2004), which would mean that winged adult insects existed about 400 million years ago. However, this does not indicate that these winged insects had terrestrial larvae or not. The concept of an earlier origin of insects at a time when the terrestrial environment was generally hostile with little vegetation, would indicate that the fossil record is far less complete than previously imagined. The hypothesis that metamorphosis evolved in amphibiotic ancestors of the winged insects is not significantly affected by any evidence for or against a hypothetical terrestrial stage ancestral to Ephemeroptera. Such a hypothesis is not considered within this paper since the explanation of metamorphosis is the same as in the aquatic model. The aquatic model provides potential answers to problems which are not explained in the terrestrial model, so it needs to be examined carefully before we accept an extra hypothetical stage in the evolution of the winged insects.

DISCUSSION

Independent adaptations to terrestrial or aquatic life?

Generally, there is agreement that insects have crossed environmental barriers from aquatic to terrestrial life or *vice versa* on numerous occasions, whether we follow an aquatic model or a terrestrial model for the evolution of insects. When an environmental barrier is crossed, such as from aquatic to terrestrial life, major adaptations have to occur. Such a transition would affect the digestive, respiratory, locomotory and circulatory systems, in addition to vision, which would all have to be significantly modified in order to allow for life within a terrestrial environment (Toms 1984*a*; Little 1990). One of the most important changes is the development of the respiratory system and this has been seen as a difficulty for the aquatic model. However, it has been suggested that the tracheal respiratory system first evolved in ancestral insects, which began to leave water for part of their life. Tracheae developed in the adult stages and their branches subsequently ramified into abdominal appendages of aquatic immatures (Riek 1971). When an environmental barrier is crossed several times in the same direction, we should find evidence of independent adaptations to the new environment, and several possible test cases have already been identified and discussed (Toms 1984*a*, 1986). Although the terrestrial model suggests that insects moved from land to fresh water on many occasions, a process which was well understood by Miall in 1895, they surprisingly seldom colonised the marine environment. In contrast, the aquatic model suggests that winged insects originated in fresh water and became terrestrial a few times, the paucity of marine insects is thus less surprising. According to both models, many habitat changes occurred in both directions providing us with an opportunity to investigate the direction of evolution and finding a way of rooting the phylogenetic tree.

Recent developments

Recent evidence shows that there are more similarities between ribosomal and mitochondrial DNA of insects and crustaceans than between insects and myriapods (Regier & Shultz 1997; Boore *et al.* 1995; Friedrich & Tautz 1995; Cameron *et al.* 2004), indicating that Myriapoda and Insecta are probably not monophyletic as was commonly assumed (Little 1990). The remarkable similarity in the construction of the brains of insects and crustaceans supports this conclusion (Osorio *et al.* 1995). This means that one of the most important assumptions which supports the theory that insects evolved from terrestrial myriapods is no longer valid and that they might have actually arisen from some ancestral aquatic Crustacea. Averof and Cohen (1997) found that homeobox genes expressed in fruit fly (*Drosophila*) wings are also expressed in crustacean exopodites or gills, suggesting that wings may be ancient appendages dating back to Crustacea, and not relatively 'recent' novelties. This discovery is significant, since it contradicts the paranotal-lobe theory for the origin of insect wings in silverfish-like ancestors and thus excludes Zygentoma as plausible ancestors of winged insects (Quartau 1985; Hasenfuss 2002). Since the hypothesis that wings are new developments has been discredited (Averof & Cohen 1997), it is proposed that the ancestors of winged insects must have had appendages from which wings could have evolved, and may have resembled primitive mayfly larvae (Kukalová-Peck 1985; Marden & Kramer 1994; Will 1995).

Ancestors of holometabolous insects

The origin of holometabolous insects is one of the key events in the evolution of winged insects. Traditional classifications assume that Holometabola are monophyletic and that their undisclosed ancestor was terrestrial (Hennig 1981). However, in the aquatic model, terrestrial holometabolous larvae may be polyphyletic. The most probable ancestral groups are Ephemeroptera, Plecoptera, Megaloptera, Trichoptera and amphibiotic Mecoptera, and the most important primitive characteristic is an aquatic life-style of their larvae.

Although several molecular phylogenies have appeared (e.g. Giribet & Ribera 2000; Wheeler *et al.* 2001; Whiting 2002; Bitsch *et al.* 2004), there is no clear evidence that terrestrial orders are primitive and there is a lack of plausible ancestors for the amphibiotic orders. Recent evidence from ribosomal DNA consistently points to a close relationship between Trichoptera and Lepidoptera (Giribet & Ribera 2000), but the larvae of Trichoptera are aquatic while those of Lepidoptera are generally terrestrial and those species with aquatic larvae are relatively specialised. The close relationship between Trichoptera and Lepidoptera is generally accepted, but the primitive state of the larvae (aquatic or terrestrial) is controversial (Hennig 1981). The egg, larva and pupal stages of the life cycle of Trichoptera are all aquatic and the adults re-enter the water to lay eggs (Morse 1997), suggesting that aquatic larvae may be primitive. Significantly, new evidence from ribosomal DNA suggests that Trichoptera consistently form a lower branch in the clade composed of Lepidoptera and Trichoptera (Giribet & Ribera 2000), pointing towards an aquatic habitat for the ancestral larvae. A similar situation exists in the case of Megaloptera (Haring & Aspöck 2004), where Megaloptera appear to be more primitive than Neuroptera and have aquatic larvae. In the aquatic model, it may be suggested that the origin of a terrestrial lifestyle in the larval stages was a major event in the origin of Neuroptera (Aspöck 2002).

Evolution of insect eyes

Another relevant development is a review of information concerning the structure of the eyes (stemmata) of holometabolous larvae (Gilbert 1994). Gilbert found that in comparison to the eyes of hemimetabolous nymphs that are similar to those of the adults, eyes of holometabolous larvae are highly reduced and very different from those of the adults. The only holometabolous larvae with vestigial compound eyes are Mecoptera (Gilbert 1994), notably the aquatic larvae of Nannochoristidae (Byers & Thornhill 1983). The Nannochoristidae have a suite of characters that are presumably primitive for Mecoptera and occupy the basal branch in Mecoptera phylogeny (Willmann 1987), and there is a trend towards the reduction of eyes from primitive aquatic species to advanced terrestrial species, as in other holometabolous larvae. In the aquatic model, the reduction in size of larval eyes could have arisen as a consequence of aquatic larval life, where the visual requirements in aquatic larvae were different from those of the terrestrial adults. The original reduction of larval eyes of holometabolous larvae must have occurred very early in their evolution, coinciding with the origin of metamorphosis, at a time when adaptation to different environments was prevalent at different stages within an amphibiotic life cycle (Toms 1984*b*). In Coleoptera, Crowson (1981) argued that reduction in numbers of stemmata from the maximum number of seven pairs (also found in some Mecoptera) is an irreversible evolutionary event, and that the number of stemmata is a good phylogenetic character since it is consistent with our understanding of Coleoptera phylogeny. It appears that Crowson's rule regarding reduction in stemmatal number may be true for all holometabolous insects if the aquatic model is valid. However, in the terrestrial model, one would have to suggest a reason for reduction of stemmata in terrestrial larvae and a reversal or increase in the number of stemmata in aquatic larvae. For example, referring to Gilbert (1994), aquatic megalopteran larvae typically have seven pairs of stemmata while most terrestrial larvae have fewer. Although neuropteran larvae primitively possess seven pairs of stemmata, the most ventral stemma is reduced in all taxa examined. In Coleoptera, the maximum number of six pairs is found in primitive aquatic adephagous larvae, while in Diptera the primitive number of stemmata (found in aquatic Nematocera larvae) is five pairs. Most of the primitive flies (Nematocera) have aquatic or semiaquatic larvae (Yeates & Wiegmann 1999), and this needs to be explained, together with the evolution of the unique respiratory systems of Diptera larvae (Clarke 1979). Such systems can be seen to reflect important apomorphic characters that may have originated as an adaptation to life in anaerobic water, allowing aquatic larvae to breathe air. In the aquatic model, the possibility that primitive Nematocera remained aquatic but developed the ability to breathe gaseous oxygen is congruent with the apparent trend from wet to drier habitats (such as fruit and dung) in fly larvae. If Diptera larvae developed the ability to breathe gaseous oxygen independently of other orders we would expect to find additional independent adaptations to terrestrial life, such as alterations in blood physiology. In fact, Diptera may have a strong dependence on Na^+ rather than Cl^- ions in their haemolymph unlike other aquatic insects where the reverse is often true (Sutcliffe 1963).

The basic number of seven pairs of lenseless stemmata is also found in Trichoptera larvae (Gilbert 1994), and these amphibiotic insects, together with Megaloptera, are regarded as primitive holometabolans within the aquatic model. In the terrestrial model, it has been seen as an anomaly that Trichoptera larvae are aquatic (specialised), while in other ways Trichoptera are more primitive than Lepidoptera (Hennig 1981). If aquatic Trichoptera larvae are advanced, it is also an anomaly that some of them have the primitive number of seven pairs of stemmata. Larvae in the primitive lepidopteran family Agathiphagidae also have seven pairs of stemmata, but in the Micropterygidae, which are sometimes regarded as the most primitive family in the terrestrial model, there are only five pairs of stemmata (most Lepidoptera larvae have six pairs of stemmata).

Testing the models

Differences in interpretation of the aquatic and terrestrial models require that tests be set up in order to refute one of the models. If aquatic insects are more primitive, the most parsimonious phylogenetic tree would have an aquatic stem with amphibiotic orders forming the basal branches from which terrestrial orders evolved. In contrast, a terrestrial model predicts that we should find a terrestrial stem with both terrestrial and aquatic branches. Terrestrial models that date back to Aristotle have failed to provide a framework capable of making verifiable predictions, and many anomalies exist. However, many observations that seem odd in the terrestrial model are anticipated in the aquatic model, which also suggests solutions to problems. For example, the difficulties experienced in explaining the origin of Hymenoptera may be a consequence of the larval stage of this group moving from aquatic to terrestrial life, which could have affected the morphology of the adult because they share the same genes. Lepidoptera may be viewed as Trichoptera with terrestrial larvae and the adaptation to terrestrial life is likely to have provided the driving force for the evolution of lungs in Lepidoptera larvae (Locke 1998) as well as a wide array of hexamerines in their highly developed haemolymph (Sutcliffe 1963; Telfer & Kunkel 1991). An interesting finding from ribosomal DNA, is that grasshoppers and crickets consistently resolve in different clades (Giribet & Ribera 2000), suggesting that the division between them is ancient and more significant than that between, for example, Trichoptera and Lepidoptera. This indicates that Kevan (1986) may have been correct in suggesting that the suborders Caelifera (grasshoppers) and Ensifera (crickets, katydids etc.) should be regarded as different orders because there are many differences between them and no shared apomorphies. Jumping legs may have evolved independently in Caelifera and Ensifera, as a result of adaptation to terrestrial life in the larval stages.

CONCLUSION

By focussing on major differences in the aquatic and terrestrial models, it should be possible to refute one of the models, establish a secure root for the insect phylogenetic tree and resolve most of the basic interrelationships between the different orders. It is a pity that data on the most interesting insects from a phylogenetic point of view, such as Megaloptera and Trichoptera, has seldom been collected. This would not have occurred if due attention had been given to the alternative predictions of the aquatic model. If evolutionary theory is used to make testable hypotheses and more attention is paid to problem solving, this will dramatically increase the rate at which progress is made.

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REFERENCES

Aspöck, U. 2002. Phylogeny of the Neuropterida (Insecta: Holometabola). *Zoologica Scripta* **31**: 51–55.

Averof, M. & Cohen, S.M. 1997. Evolutionary origin of insect wings from ancestral gills. *Nature* **385**: 627–630.

Byers, G.W. & Thornhill, R. 1983. Biology of the Mecoptera. *Annual Review of Entomology* **28**: 203–228.

Bitsch, J., Bitsch, C., Bourgoin, T. & D'Haese, C. 2004. The phylogenetic position of early hexapod lineages: morphological data contradict molecular data. *Systematic Entomology* **29**: 433–440.

Boore, J.L., Collins, T.M., Stanton, S., Daehier, L.L. & Brown, W.M. 1995. Deducing the pattern of arthropod phylogeny from mitochondrial DNA rearrangements. *Nature* **376**: 163–165.

Cameron, S.L., Miller, K.B., D'Haese, C.A., Whiting, M.F. & Barker, S.C. 2004. Mitochondrial genome data alone are not enough to unambiguously resolve the relationships of Entognatha, Insecta and Crustacea *sensu lato* (Arthropoda). *Cladistics* **20**: 534–557.

Carpenter, F.M. 1992. *Treatise on Invertebrate Paleontology*. Part R, Arthropoda 4, Volume 3. Boulder, Colorado and Lawrence, Kansas: Geological Society of America and University of Kansas.

Caterino, M.S., Cho, S. & Sperling, F.A.H. 2000. The current state of insect molecular systematics: a thriving Tower of Babel. *Annual Review of Entomology* **45**: 1–54.

Clarke, K.U. 1979. Visceral anatomy and arthropod phylogeny. *In*: Gupta, A.P., ed., *Arthropod Phylogeny*. New York: Van Nostrand Reinhold, pp. 467–549.

Crampton, G.C. 1918. The probable ancestors of insects and myriopods. *Canadian Entomologist* **50**: 285–288.

Crowson, R.A. 1981. *The Biology of the Coleoptera*. London: Academic Press.

Engel, M.S. & Grimaldi, D.A. 2004. New light shed on the oldest insect. *Nature* **427**: 627–630.

Friedrich, M.F. & Tautz, D. 1995. Ribosomal DNA phylogeny of the major extant arthropod classes and the evolution of myriapods. *Nature* **376**: 165–167.

Gegenbaur, C. 1874. *Grundriss der vergleichenden Anatomie*. Leipzig: Engelman.

Gilbert, C. 1994. Form and function of stemmata in larvae of holometabolous insects. *Annual Review of Entomology* **39**: 323–349.

Giribet, G. & Ribera. C. 2000. A review of arthropod phylogeny: new data based on ribosomal DNA sequences and direct character optimization. *Cladistics* **16**: 204–231.

Hansen, H.J. 1893. Zur Morphologie der Gliedmassen und Mundtheile bei Crustaceen und Insekten. *Zoologischer Anzeiger* **16**: 193–198, 201–212.

Haring, E. & Aspöck, U. 2004. Phylogeny of the Neuropterida: a first molecular approach. *Systematic Entomology* **29** (3): 415–430.

Hasenfuss, I. 2002. A possible evolutionary pathway to insect flight starting from lepismatid organization. *Journal of Zoological Systematics and Evolutionary Research* **40**: 65–81.

Hennig, W. 1981. *Insect Phylogeny*. New York: John Wiley.

Kevan, D.K. McE. 1986. A rationale for the classification of orthopteroid insects—the saltatorial orthopteroids or grigs—one order or two? *Proceedings of the 4th Triennial Meeting of the Pan American Acridological Society* **4**: 49–67.

Krell, F.-T. & Cranston, P.S. 2004. Which side of the tree is more basal? *Systematic Entomology* **29**: 279–381.

Kukalová-Peck, J. 1978. Origin and evolution of insect wings and their relation to metamorphosis, as documented by the fossil record. *Journal of Morphology* **156**: 53–126.

———1985. Ephemeroid wing venation based upon new gigantic Carboniferous mayflies and basic morphology, phylogeny and metamorphosis of pterygote insects (Insecta, Ephemerida). *Canadian Journal of Zoology* **63**: 933–955.

Little, C. 1990. *The Terrestrial Invasion*. Cambridge: Cambridge University Press.

Locke, M. 1998. Caterpillars have evolved lungs for haemocyte gas exchange. *Journal of Insect Physiology* **44**: 1–20.

Marden, J.H. & Kramer, M.G. 1994. Surface-skimming stoneflies: a possible intermediate stage in insect flight evolution. *Science* **266**: 427–430.

Miall, L.C. 1895. *The Natural History of Aquatic Insects*. London: Macmillan.

Morse, J.C. 1997. Phylogeny of Trichoptera. *Annual Review of Entomology* **42**: 427–450.

Osorio, D., Averof, M. & Bacon, J.P. 1995. Arthropod evolution: great brains, beautiful bodies. *Trends in Ecology and Evolution* **10**: 449–454.

Pritchard, G., McKee, M.H., Pike, E.M., Scrimgeour, G.J. & Zloty, J. 1993. Did the first insects live in water or in air? *Biological Journal of the Linnean Society* **49**: 31–44.

Quartau, A.J. 1985. On some objections to the paranotal theory on the origin of the insect wings. *Boletim da Sociedade Portuguesa de Entomologia* **Suppl. 1** (2): 259–371.

REGIER, J.C. & SHULTZ, J.W. 1997. Molecular phylogeny of the major arthropod groups indicates polyphyly of crustaceans and a new hypothesis for the origin of hexapods. *Molecular Biology and Evolution* **14**: 902–913.

REIK, E.F. 1971. The origin of insects. *In*: *Proceedings of XII International Congress of Entomology, Moscow 2-9 August, 1968*. Volume 1. Leningrad, Moscow: USSR Academy of Sciences, pp. 292–293.

SHCHERBAKOV, D.E. 1999. Controversies over the insect origin revisited. *In*: *Proceedings of the First International Palaeoentomological Conference, Moscow, 1998.* Bratislava: AMBA Projects, pp. 141–148.

SINITSHENKOVA, N.D. 2002. Ecological history of the aquatic insects. *In*: Rasnitsyn, A.P. & Quicke, D.L.J., eds., *History of Insects*. Dordrecht *etc.*: Kluwer Academic Publishers, pp. 388–426.

SUTCLIFFE, D.W. 1963. The chemical composition of haemolymph in insects and some other arthropods in relation to their phylogeny. *Comparative Biochemistry and Physiology* **9**: 121–153.

TELFER, W.H. & KUNKEL, J.G. 1991. The function and evolution of insect storage hexamers. *Annual Review of Entomology* **36**: 205–228.

TOMS, R.B. 1984*a*. Were the first insects terrestrial or aquatic? *South African Journal of Science* **80**: 319–323.

———1984*b*. Environmental selective pressures and the origin of insect metamorphosis. *Journal of the Entomological Society of southern Africa* **47**: 269–276.

———1985. Hypothetical gaps in the insect fossil record. *Annals of the Geological Survey of South Africa* **19**: 89–93.

———1986. A synopsis of explanations for insect and amphibian metamorphosis. *South African Journal of Science* **82**: 372–375.

TRUMAN, J.W. & RIDDIFORD, L.M. 1999. The origins of insect metamorphosis. *Nature* **401**: 447–452.

WHEELER, W.C., WHITING, M., WHEELER, Q.D. & CARPENTER, J.M. 2001. The phylogeny of the extant Hexapoda orders. *Cladistics* **17**: 113–169.

WHITING, M.F. 2002. Phylogeny of the holometabolous insect orders: molecular evidence. *Zoologica Scripta* **31** (1), 3–15.

WILL, K.W. 1995. Plecopteran surface-skimming and insect flight evolution. *Science* **270**: 1684–1685.

WILLMANN, R. 1987. The phylogenetic system of the Mecoptera. *Systematic Entomology* **12**: 519–524.

———1997. Advances and problems in insect phylogeny. *In*: Fortey, R.A. & Thomas, R.H., eds, *Arthropod relationships*. London: Chapman & Hall, pp. 269–279.

YEATES, D.K. & WIEGMANN, B.M. 1999. Congruence and controversy: toward a higher-level phylogeny of Diptera. *Annual Review of Entomology* **44**: 397–428.

ZHERIKHIN, V.V. 2002. Ecological history of the terrestrial insects. *In*: Rasnitsyn, A.P. & Quicke, D.L.J., eds, *History of Insects*. Dordrecht *etc.*: Kluwer Academic Publishers, pp. 331–388.